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Molecular docking and ADME studies of natural compounds of *Centella asiatica* for antiepileptic activity

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Abstract
$GABA_A$ receptors that are activated upon binding to γ-aminobutyric acid (GABA), an inhibitory neurotransmitter, where they play a role in epilepsy. Targeting $GABA_A$ receptors through specific enhancement of neuronal inhibition involving GABA can be a better mechanism for identification of novel antiepileptic drugs. In this study, screening of novel lead candidates from the plant Centella asiatica (CA) against human GABRB3 (4COF) active site using molecular docking. The docking analysis done reveals the identification of leads with favorable binding energy and hydrogen bond interactions confirmed the effective modulation of the receptor. Based on the dock score and number of hydrogen bond interactions, compound Centelloside b observed to be the most potent compound. Our ADMET studies allow us to evaluate these CA derived compounds and to assess the parameter that will be essential for further lead optimization efforts.

Keywords: *Centella asiatica*, ADME, epilepsy, $GABA_A$ receptors, docking studies

Introduction
Epilepsy is a ceaseless and commonly persuasive neurological issue symbolized by the intermittent and uncertain episodes of epileptic seizures that are due to the exceptional cerebral neuronal release that consequence in almost instantaneous protest of sensation and loss of consciousness. Epileptic seizures can influence a range of symptoms confide in the brain distressed areas which may vary from mild to severe incorporating complete or partial loss of consciousness, loss of speech, uncontrollable motor behavior and unusual sensory experiences [1, 7]. This neurological disorder has been one of the most researched medical conditions mainly due to its complex morbidity associations with substantially high mortality rates [8, 9]. Epilepsy, being the top three acclaimed contributors to global burden of neurological disorders, arousing affect around 65 million people worldwide besides extensively varying its prevalence and incidence from 2.8 to 19.5 per 1000 of the general population all through the world [10]. The cause of most cases of epilepsy is unknown· Some cases occur as the result of brain injury, stroke, brain tumours, infections of the brain, and birth defects, through a process known as epileptogenesis [11, 12, 13]. Known genetic mutations are directly linked to a small proportion of cases. The acceptable pathogenesis of epileptic seizures is the imbalance of excitatory and inhibitory neurotransmitters in central nervous system, which lead an abnormal nerve cell activity and neuronal discharge resulting in seizures. Thus, reduction in duration, as well as the onset of seizures by modulating these neurotransmitters, was the main strategy for epilepsy treatment [14, 15]. The desire for pharmacological manipulation of GABAergic neurotransmission has generated a plethora of xenobiotics which are useful in medicine, including anticonvulsants, anesthetics, anxiolytics, muscle relaxants and medications for treating pain [16].

GABA (γ-Aminobutyric acid), the major inhibitory neurotransmitter in vertebrate central nervous system (CNS), exerts its action primarily by activating the $GABA_A$ receptors ($GABA_A$Rs). GABA molecules or GABA-like compounds bind to the receptor and activate it. Upon activation, the GABA-A receptor selectively conducts Cl^- through its pore, resulting in hyperpolarization of the neuron. This causes an inhibitory effect on neurotransmission by diminishing the chance of a successful action potential [17, 18, 19]. This cumulative neuronal inhibition caused by GABA binding to many neurons results anticonvulsive properties [20]. GABAA receptors are constituted by a pseudo symmetrical assembly of five identical or homologous subunits forming a chloride-conducting ion pore [21, 24]. Each subunit comprises a 200 to 250 amino acids long extracellular N-terminal domain, a loose bundle of four membrane-spanning α-helices (TM1-TM4), a large intracellular loop between the TM3 and

TM4 domain (between 85 and 255 amino acid residues) and a short C-terminal segment. Residues from the TM2 domain line the ion-conducting pore. The realization that the GABAA receptor system is a collage derived from 6α, 3β, 3γ, δ, θ, ε, π and 3ρ subunits 2, 3 and that different recombinant subunits are particularly important in certain physiologic events mediated by GABA. This has generated GABAA receptors with distinct electrophysiological along with pharmacological properties including agonist sensitivity as well as its sensitivity for drugs. Furthermore, these distinct receptor subtypes stimulated a search for chemical entities that have selectivity for GABAA receptors with a particular combination of subunits [25, 28].

Although seizures are controlled with currently available AEDs but more than 30% patients still have medically refractory epilepsy. Moreover, about 30-40% epileptic patients are still affected by many side effects. These conditions have motivated the researchers to develop novel approaches to treat epilepsy like antiepileptic constituents from herbal medicines [29, 30, 31]. Gotu Kola bearing a scientific name as Centella asiatica (CA) and belongs to Apiaceae (Umbelliferae) family is an herbaceous annual plant which is native to Asia. The vital bioactive components of C. asiatica leaf are saponin glycosides (Brahmiside, Brahminoside), triterpenoid glycosides (asiatic acid, medacassic acid) and flavonoids. The extract of Gotu kola was documented to be beneficial in developing memory and additionally for the treatment of anxiety and mental fatigue. CA aids to reverse the effect of GABAA antagonist, PTZ and guard against PTZ-induced convulsions and the inhibition of ATPase [32, 40]. By taking this diversity of ligands from plant origin of CA differing structurally from synthetic GABAA receptor modulators, our present study initiates a methodology aiming at the discovery of these compounds as GABAA receptor agonists as the targets of epilepsy. We are interested to study the possible interactions of CA derived compounds with the first reported three-dimensional structure of a GABA(A)R, the human β3 homopentamer, at 3 Å resolution (PDB ID: 4COF). Further docking with the selected CA derived compounds is done and the analysed results show the increased response of the ligands towards the receptor. From the studies it was revealed that the -compound Centelloside b having highest potentiality to act as potent leads in modulation of GABA(A)R receptor in the treatment of epilepsy.

Materials and methods

Protein preparation

The Crystal structure of a human gamma-aminobutyric acid receptor, the GABA (A)R-beta3 homopentamer (GABRB3) was retrieved from Protein Data Bank (PDB) with PDB ID: 4COF. The X-Ray diffraction structure of this receptor had a resolution of 2.97 Å, R value of 0.206 and R free value of 0.226. In the first step, the protein preparation protocol of Discovery Studio (DS) was used to prepare the protein structure retrieved from the PDB. The water molecules and the hetero atom were removed. Hydrogen atoms were added to the protein structures corresponding to pH value of 7.4. Then the protocol performs protein structure refinement that corrects their bond orders, modeling missing loop regions, inserting missing atoms in incomplete residues, deleting alternate conformations, standardizing names of the atoms and protonating titratable residues. Finally, all-atom restrained energy minimization of the protein structure was carried out using CHARMm force field with steepest descent algorithm followed by conjugant gradient algorithm until the convergence gradient satisfied with a root mean square deviation (RMSD) tolerance of 0.01 Å. After energy minimisation, Using Define and Edit Binding Site tools in DS, the active site of the protein was selected based on the bound ligand benzamidine conformation and a active site sphere was defined with a radius of 10 Å respectively.

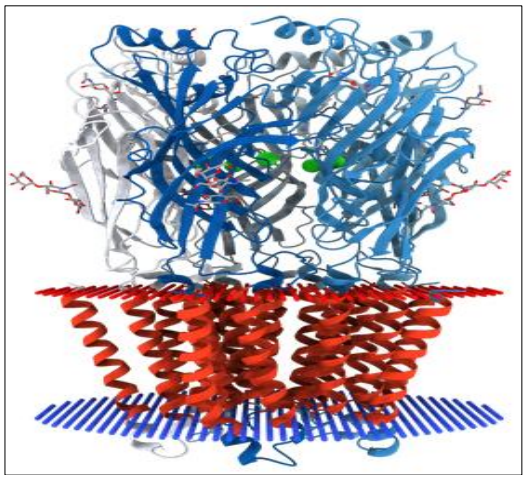

Fig 1: The Crystal Structure of Aspirin a human gamma-aminobutyric acid receptor, the GABA (A)R-beta3 homopentamer (PDB ID: 4COF).

Ligand preparation and optimization

The CA derived compounds chosen in the present study were Aglycone, Asiaticoside, Centelloside b and Scheffoleoside A. The chemical structures of the compounds are sketched using ACD/ChemSketch (12.0) Software and saved in mol2 format. These ligands were then subjected to prepare ligands protocol of DS. They were converted from 2D to 3D structures by including stereo chemical, ionization, tautomeric variations, as well as energy minimization and optimized for their geometry, desalted and corrected for their chiralities and missing hydrogen atoms. The bonds orders of these ligands were fixed and the charged groups were neutralized. The ionization and tautomeric states were generated between pH of 6.8 to 7.2. In the final stage of Ligand preparation, compounds were minimized using CHARMm force field until a root mean square deviation of 0.01 was achieved. Steepest descent algorithm was used for minimization, followed by conjugate gradient method. A single low energy confirmation per ligand was generated and the optimized ligands were used for docking analysis.

Molecular docking studies

The molecular docking studies were performed with the help of the DS LibDock program which estimates the appropriate binding conformations of compounds in the defined active site of human GABRB3 (PDB ID: 4COF).. The LibDock is a flexible docking module. LibDock uses protein site features, referred to as hot spots, consisting of two types states (polar and apolar). The ligand poses are placed into the polar and apolar receptor interactions site. A polar hotspot is preferred by a polar ligand atom (e.g., a hydrogen bond donor or acceptor), and an apolar hotspot is preferred by an apolar atom (e.g., a carbon atom). The protocol allows the user to specify several modes for generating ligand conformations for docking. Scoring function of the LibDock calculates the binding affinity score or docking score (LibDock score) of protein-ligand complex. Also the possible binding energies,

possible hydrogen bonding and various interaction poses are calculated. The top ranked docked complexes of each compound are selected on the basis of LibDock Score. Binding poses with highest LibDock Score and lowest binding energy are preferred as the best pose and further binding interactions of the best pose for each compound are analyzed.

In silico pharmacokinetics studies

Different pharmacokinetics parameters, namely, Absorption, Distribution, Metabolism, Excretion, and Toxicity were calculated. ADMET profiling of compounds were performed by applying ADMET descriptors protocol of DS. This study includes the quantitative measurement of drug-like properties include aqueous solubility, blood brain barrier (BBB), plasma protein binding (PPB), CYP2D6 binding, intestinal absorption and hepatotoxicity. In addition, AlogP98 and PSA_2D were used in plotting the confidence ellipses. Analysis of the results is based on the standard parameters according to the software limitations.

Results and discussion

Docking studies

Molecular docking study was performed for the CA compounds using the human GABRB3 (PDB ID: 4COF) enzyme as receptor molecule with the aid of Libdock module of DS. Binding affinity evaluation and inhibitory potential of these compounds were measured through LibDock docking score and H-bond interactions. Of all the conformations generated for each compound, the compound with the highest LibDock score is taken for interaction analysis of the hydrogen bonding. The hydrogen bond interaction is significant for the bioactivity of compounds. The stability of the best docked pose of these compounds was evaluated by determining the hydrogen bonding interactions of the protein with compounds which revealed the critical amino acids involved in hydrogen bond formation. The high LibDock score of the ligand pose with least binding energy was taken into account for the prediction of the best ligand binding conformation. Apart from hydrogen bonding interactions, other non-bonded interactions like hydrophobic bonding were also observed. Table 1 depicts the LibDock scores, interaction data and binding energies for CA compounds.

Docking results showed that LibDock program successfully docked four compounds namely Aglycone, Asiaticoside, Centelloside b and Scheffoleoside A into the binding site of human GABRB3 (PDB ID: 4COF) with LibDock scores of 76.158, 130.267, 116.635 and 97.634. After scrutinizing all the results of docking and interaction analysis, the best docking score of for compound Asiaticoside was achieved against the human GABRB3 receptor forming a single hydrogen bond with the amino acid residue TYR157. The compound Asiaticoside showed a high binding affinity as indicated by LibDock score of 130.267 and high binding energy of 2884.5483 kcal/mol but considering the hydrogen bonding interactions are very less inferring low stability. Next to Asiaticoside, the compound Centelloside b has shown a LibDock score of 116.635 forming three hydrogen bonding interactions with lowest binding energy of 97.56713. The compounds Aglycone and Scheffoleoside A having low docking scores and hydrogen bond interactions than the compound Centelloside b inferring lower binding affinity and stability. Based on these factors, among all the compounds Centelloside b was considered as the best compound indicating high binding affinity and better hydrogen bond interactions with the human GABRB3 receptor active site residues. The human GABRB3 (PDB ID: 4COF) and Centelloside b interaction visualization is shown in the Figure 2. From the Figure 2 it is observed that compound Centelloside b formed two hydrogen bonds with TYR157 and a single hydrogen bond with TYR97. A hydrogen bond is formed when the hydrogen atom of TYR97 interacted with the 15th oxygen atom of the compound Centelloside b (A:TYR97:HH - Centelloside b:O15) with a hydrogen bond distance of 1.858 Å. The oxygen atom and hydrogen atom of the residue TYR157 interacts with 69th H-atom and 7th oxygen of the compound forming two hydrogen bonds (Centelloside b:H69 - A:TYR157:O and A:TYR157:HH - Centelloside b:O7) with a hydrogen bond distances of 2.108 Å and 2. 088 Å respectively. Some non-bonded interactions are found between the compound Centelloside b and the residues Ser156, Glu155 and Phe200.

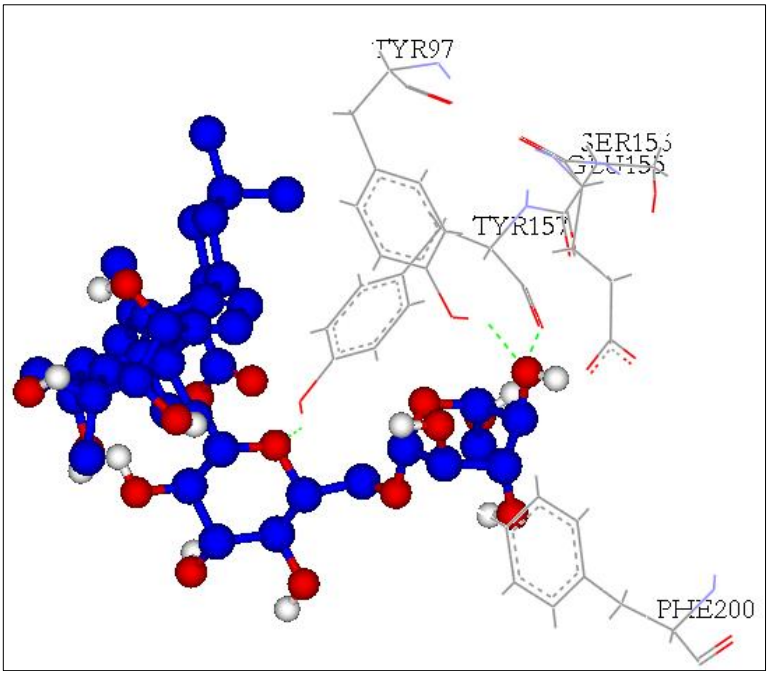

Fig 2: Receptor-ligand Hydrogen bonding interactions (green color) of Centelloside b.

Table 1: Calculated docking scores, binding energies and interaction atoms along with their bond lengths of the targeted compounds inside *human* GABRB3 (4COF) active site

Name	Libdockscore	Interacting amino acids	Interacting atoms	H-distance (Aº)
Aglycone	76.158	Tyr97, Ser156 Glu155, Tyr157 Phe200	A:TYR97:HH - Aglycone:O2 Aglycone:H34 - A:TYR97:OH Aglycone:H31 - A:PHE200:HE2 Aglycone:H31 - A:PHE200:CE2 A:TYR97:HH - Aglycone:H43	2.408000 2.471000 1.669000 2.205000 1.526000
Asiaticoside	130.267	Tyr97, Ser156 Glu155, Tyr157 Phe200	Asiaticoside:H79 - A:TYR157:O Asiaticoside:O19 - A:TYR157:O Asiaticoside:C39 - A:TYR97:HB2 Asiaticoside:C67 - A:PHE200:HE2	2.478000 2.240000 2.161000 2.126000
Centelloside b	116.635	Tyr97, Ser156 Glu155, Tyr157 Phe200	A:TYR97:HH - Centelloside b:O15 Centelloside b:H69 - A:TYR157:O A:TYR157:HH - Centelloside b:O7	1.858000 2.108000 2.088000
Scheffoleoside A	97.634	Tyr97, Ser156 Glu155, Tyr157 Phe200	Scheffoleoside A:H76-A:TYR157:OH A:TYR157:HE2-Scheffoleoside A:C59	2.408000 1.956000

In silico pharmacokinetics studies

ADMET studies of CA compounds predicted using ADMET descriptor module of DS to provide insight into the pharmacokinetic property of the compounds. All the parameters calculated are tabulated in the Table 2. According to the Discovery Studio parameters, standard analysis value like level 0 for human intestinal absorption, level 3 and level 4 for solubility, level 0 for non-inhibitory property with CYP450 2D6, level 3 for BBB penetration and level 0 for non-toxicity were filtered for obtaining drug like compounds. ADMET descriptors, the 2D polar surface area in A2 per compound are plotted against their consonant estimated atom-type partition coefficient (ALogP98). The space covered by the ellipse is a prophecy of excellent absorption without any violation of ADMET properties. Ellipses indicate the absorption model at 95% and 99% confidence limit to the BBB and intestinal absorption models. The plot of polar surface area and ALogP for CA compounds are represented in Figure 3.

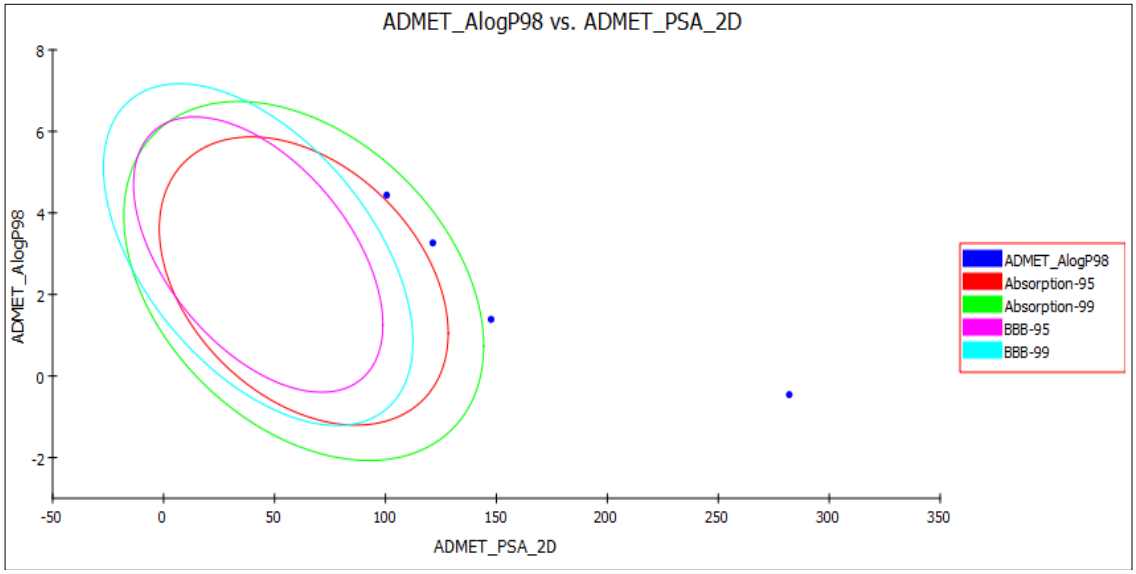

Fig 3: Plot of PSA versus LogP for candidate compounds showing the 95 and 99% confidence limit ellipses corresponding to the blood–brain barrier and intestinal absorption models

Table 3: predicted ADMET values of CA derived compounds.

	Name	ADMET_BBB_L	ADMET_Absorpti	ADMET_Solubility	ADMET_Hepatotc	ADMET_CYP2D6	ADMET_PPB_Leve	ADMET_AlogP98	ADMET_PSA_2D
1	Aglycone	4	2	-2.994	1	0	1	1.39	147.609
2	Asiaticoside	4	3	-6.113	1	0	0	-0.101	320.667
3	Centelloside b	4	3	-5.424	1	0	0	-0.457	281.991
4	Scheffoleoside A	4	3	-6.092	1	0	0	-0.146	320.667

Conclusion

In conclusion, the modulation of GABAA receptors via stimulating GABA mediated neuronal inhibition, promises to be a favorable therapeutic approach in the treatment of epilepsy. The present study initiates an attempt to find the potential compound from the plant Centella asiatica. The molecular docking study on human GABRB3 (4COF) confirmed the active modulation by Centella asiatica derived compounds. The compound Centelloside b can acts as specific leads for receptor modulation and assist in discovery of novel anti-epileptic drugs.

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